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McGuire, William orcid.org/0000-0001-8572-3467 (2019) Enteral lactoferrin supplementation for very preterm infants : a randomised controlled trial. Health technology assessment. ISSN 2046-4924

[https://doi.org/10.1016/S0140-6736\(18\)32390-0](https://doi.org/10.1016/S0140-6736(18)32390-0)

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Enteral lactoferrin supplementation for very preterm infants: a randomised placebo-controlled trial

The ELFIN trial investigators group*



Summary

Background Infections acquired in hospital are an important cause of morbidity and mortality in very preterm infants. Several small trials have suggested that supplementing the enteral diet of very preterm infants with lactoferrin, an antimicrobial protein processed from cow's milk, prevents infections and associated complications. The aim of this large randomised controlled trial was to collect data to enhance the validity and applicability of the evidence from previous trials to inform practice.

Methods In this randomised placebo-controlled trial, we recruited very preterm infants born before 32 weeks' gestation in 37 UK hospitals and younger than 72 h at randomisation. Exclusion criteria were presence of a severe congenital anomaly, anticipated enteral fasting for longer than 14 days, or no realistic prospect of survival. Eligible infants were randomly assigned (1:1) to receive either enteral bovine lactoferrin (150 mg/kg per day; maximum 300 mg/day; lactoferrin group) or sucrose (same dose; control group) once daily until 34 weeks' postmenstrual age. Web-based randomisation minimised for recruitment site, gestation (completed weeks), sex, and single versus multifetal pregnancy. Parents, caregivers, and outcome assessors were unaware of group assignment. The primary outcome was microbiologically confirmed or clinically suspected late-onset infection (occurring >72 h after birth), which was assessed in all participants for whom primary outcome data was available by calculating the relative risk ratio with 95% CI between the two groups. The trial is registered with the International Standard Randomised Controlled Trial Number 88261002.

Findings We recruited 2203 participants between May 7, 2014, and Sept 28, 2017, of whom 1099 were assigned to the lactoferrin group and 1104 to the control group. Four infants had consent withdrawn or unconfirmed, leaving 1098 infants in the lactoferrin group and 1101 in the sucrose group. Primary outcome data for 2182 infants (1093 [99·5%] of 1098 in the lactoferrin group and 1089 [99·0%] of 1101 in the control group) were available for inclusion in the modified intention-to-treat analyses. 316 (29%) of 1093 infants in the intervention group acquired a late-onset infection versus 334 (31%) of 1089 in the control group. The risk ratio adjusted for minimisation factors was 0·95 (95% CI 0·86–1·04; $p=0·233$). During the trial there were 16 serious adverse events for infants in the lactoferrin group and 10 for infants in the control group. Two events in the lactoferrin group (one case of blood in stool and one death after intestinal perforation) were assessed as being possibly related to the trial intervention.

Interpretation Enteral supplementation with bovine lactoferrin does not reduce the risk of late-onset infection in very preterm infants. These data do not support its routine use to prevent late-onset infection and associated morbidity or mortality in very preterm infants.

Funding UK National Institute for Health Research Health Technology Assessment programme (10/57/49).

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Introduction

Late-onset infection (occurring >72 h after birth) is the most common serious complication associated with hospital care for preterm infants. The James Lind Alliance Preterm Birth Priority Setting Partnership has identified the development and assessment of improved methods to prevent infection in preterm infants as a research priority.¹

The prevalence of late-onset infection is estimated to be higher than 20% in very preterm infants (ie, born before 32 weeks' gestation) reflecting their duration of exposure to invasive procedures and intensive care.² Very preterm infants who acquire a late-onset infection have an

increased risk of mortality and acute morbidities, including necrotising enterocolitis, retinopathy of prematurity, and bronchopulmonary dysplasia.³ Late-onset infection is associated with adverse neurodevelopmental outcomes including visual, hearing, and cognitive impairment, and cerebral palsy.⁴ Very preterm infants with late-onset infection spend a longer time in hospital than similar infants without infection.⁵ Late-onset infection has major consequences for health-care service management, delivery, and costs.

Lactoferrin, a member of the transferrin family of iron-binding glycoproteins, is a key component of the mammalian innate response to infection. It is the major

Published Online
January 8, 2019
[http://dx.doi.org/10.1016/S0140-6736\(18\)32221-9](http://dx.doi.org/10.1016/S0140-6736(18)32221-9)
See Online/Comment
[http://dx.doi.org/10.1016/S0140-6736\(18\)32390-0](http://dx.doi.org/10.1016/S0140-6736(18)32390-0)

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See Online for appendix

Research in context

Evidence before this study

The 2011 version of the Cochrane review of enteral lactoferrin supplementation in very preterm infants included one randomised controlled trial (RCT) done in 11 Italian neonatal centres. This trial, in which 331 infants participated, suggested that enteral supplementation with bovine lactoferrin reduced the incidence of late-onset infection by two thirds compared with controls. No evidence of an effect on mortality was found and no adverse effects or intolerance were reported. Because the effect size estimate might have been inflated by performance and detection bias and by other methodological weaknesses, the Cochrane review concluded that this evidence was insufficient to support a change in practice.

Added value of this study

The ELFIN trial shows that enteral supplementation of bovine lactoferrin (150 mg/kg per day until 34 weeks postmenstrual age) does not reduce the risk of late-onset infection, other morbidity, or mortality in very preterm infants. This is the largest trial of this intervention and the validity is enhanced by

the methodological quality and power. The findings are broadly applicable to very preterm infants cared for in facilities in well resourced health services. There was no indication of any beneficial effects for infants with additional risk factors for late-onset infection, including extreme preterm birth

Implications of all the available evidence

The ELFIN trial findings contradict the existing evidence base and illustrate why high-quality evidence from adequately powered RCTs is needed to inform policy and practice. The 2017 update of the Cochrane review includes six RCTs, but most were small and some contained other design and methodological weaknesses that might have introduced biases resulting in overestimation of the effect sizes. With more than twice as many participants than had been enrolled in all of the previous trials combined, the ELFIN trial generated more precise estimates of effect, and provides valid and applicable evidence that enteral bovine lactoferrin supplementation does not reduce the risk of late-onset infection or other morbidity or mortality in very preterm infants.

they protein in human breast milk, present at a concentration of about 1 mg/mL. Lactoferrin has broad microbicidal activity by mechanisms such as cell-membrane disruption, iron sequestration, inhibition of microbial adhesion to host cells, and prevention of biofilm formation.⁶ Lactoferrin also promotes the growth of probiotic bacteria, stimulates differentiation and proliferation of enterocytes and expression of intestinal digestive enzymes, and has direct intestinal immunomodulatory and anti-inflammatory actions including suppression of free-radical activity.^{7,8}

Very preterm infants typically ingest little or no milk during the early neonatal period and have low intake of lactoferrin.⁹ The low lactoferrin intake in the early neonatal period is exacerbated by any delays in establishing enteral feeding. Enteral supplementation with bovine lactoferrin has been proposed as a simple strategy to compensate for this immunodeficiency.¹⁰ Bovine lactoferrin is about 70% homologous with human lactoferrin but has higher antimicrobial activity.¹¹ It is inexpensive compared with human or recombinant lactoferrin, is available commercially as a food supplement in a stable powder form, and is “generally recognised as safe” by the US Food and Drug Administration.¹² The no-observed-adverse-effect level is more than 2 g/kg per day in rats.¹³ The European Food Safety Authority Panel concludes that bovine lactoferrin is safe for infants at the standard supplementation concentration (up to 210 mg/kg of bodyweight per day).¹⁴

The 2017 Cochrane review¹⁵ of enteral lactoferrin supplementation for preterm infants includes six randomised controlled trials (RCTs) involving 1071 participants in total. Meta-analyses suggest that lactoferrin supplementation reduces the risk of late-onset infection by

about 40%. The risk of necrotising enterocolitis was found to decrease by about 60%. No adverse effects or intolerance were reported. Since the included trials were small and contained methodological weaknesses associated with selection and performance bias, and since meta-analyses were limited by data availability and heterogeneity, the assessment of the Grading of Recommendations Assessment, Development and Evaluation working group of the quality of this evidence was low. The authors of the Cochrane review¹⁵ concluded that data from large, good-quality RCTs of lactoferrin supplementation in very preterm infants were needed to enhance the validity and applicability of the evidence to inform practice.

The aim of the Enteral Lactoferrin in Neonates (ELFIN) trial was to provide those data.¹⁶

Methods

Study design

This randomised, parallel-group, placebo-controlled trial was done in neonatal units in the UK (37 recruiting sites and 97 continuing care sites; appendix). The trial protocol was approved by the National Research Ethics Service Committee East Midlands—Nottingham 2 (Ref: 13/EM/0118). The protocol is available at the trial website and is published in summary.¹⁷ Protocol changes are listed in the appendix. Local approval and site-specific assessments were obtained from National Health Service Trusts for trial sites.

Participants

Eligible participants were very preterm infants who were younger than 72 h at randomisation. Exclusion criteria were presence of a severe congenital anomaly, anticipated enteral fasting for longer than 14 days, or no realistic

prospect of survival. Written consent was sought from the parents after they had received a full verbal and written explanation of the trial. Parents who did not speak English were approached only if an adult interpreter was available. Trial participants or their parents were not offered any material incentive or compensation to take part. Parents remained free to withdraw their infant from the trial at any time without providing a reason or explanation. Parents were aware that this decision would have no impact on any aspects of their infant's care.

Randomisation

To confirm participant eligibility, investigators supplied gestational age, sex, and time of birth. To proceed to randomisation, investigators confirmed that signed informed consent was available. Infants were randomly assigned (1:1) to receive either bovine lactoferrin (lactoferrin group) or sucrose (control group) using a minimisation algorithm to ensure balance between the groups in recruiting site (neonatal unit), sex, single versus multiple births, and gestational age in completed weeks. Twins or higher-order multiple birth infants were randomly allocated individually. Infants were considered to have been enrolled once they had been given a unique study number and allocated a treatment pack identification number. Allocation of participants was done via a secure web-based randomisation facility at the National Perinatal Epidemiology Unit (NPEU), University of Oxford. Telephone assistance and randomisation back-up was available at all times. Parents, clinicians, investigators, and outcome assessors were masked to group assignment. Clinicians could unmask a participant's allocation via the randomisation website using a single-use access code provided in a sealed envelope. The reason for unmasking had to be recorded. Unmasking was only permitted if it was an emergency situation in which knowledge of treatment allocation was needed to guide the clinical management of the participant.

Dry bovine lactoferrin has a pale pink-brown tinge whereas sucrose was very light brown. The opaque containers used to store the products did not allow them to be seen unless the sealed stopper was removed intentionally. The lactoferrin powder had similar granularity to sucrose so that when—before the addition of water and milk (see further details in the procedures section)—either product was shaken within the opaque, sealed pots it was not possible to distinguish lactoferrin from sucrose by the sound generated. Mixing the lactoferrin with sterile water and milk (either breast milk or formula) generated foam that settled within 30 min after shaking. When the mixed lactoferrin was removed in a syringe with a purple plunger, the pink tinge to the lactoferrin was disguised by the colour of the milk which often resulted in a light brown colour, which varied markedly between batches of milk. As lactoferrin was

more likely than sucrose to retain a light pink tinge, we supplied all sites with a laminated picture of a range of possible colours for the lactoferrin mixture in syringes, and stressed that this applied to both lactoferrin and sucrose.

Procedures

Trial participants were allocated to receive either bovine lactoferrin (Tatua Co-operative Dairy Company Ltd, Morrinsville, New Zealand) or sucrose (British Sugar, Peterborough, UK).

The investigational medicinal product (IMP) was processed at the UK Medicines and Healthcare Regulatory Authority (MHRA)—approved National Health Service clinical-trials pharmacy unit at the Royal Victoria Hospital, Newcastle-upon-Tyne, UK (MHRA Manufacturer's Authorisations for IMP 17136). The lactoferrin manufacturer's certificate of analysis confirmed absence of contamination from bovine transmissible spongiform encephalopathies, as per European Medicines Evaluation Agency standards. The pharmacy independently confirmed IMP purity and uniformity, sterility, low humidity, temperature stability, and processing as per good manufacturing practice standards before the batch was released by the designated qualified person.

The IMP was packaged in individual doses (375 mg) in 25 mL sealed, opaque pharmacy pots and assembled into participant packs. Boxes containing 24 identically numbered pots were labelled with the same pack identification number to indicate that they belonged to the same treatment course. At randomisation, the study number was added to the label of the allocated pack with the infant's name and date of birth for checking before each administration of the IMP.

The IMP was prescribed at a dose of 150 mg/kg bodyweight per day (up to a maximum of 300 mg/day) and prepared for administration by addition by syringe of sterile water (4 mL) plus expressed breast milk or formula (1 mL). The pot was shaken vigorously by hand for 30 s to generate a mixture containing 75mg/mL of IMP. The mixture was allowed to stand for 30 min before removal for administration using an enteral feeding syringe.

The IMP was administered once daily via gastric tube once the infant's enteral feed volume was more than 12 mL/kg per day and continued until 34 weeks' postmenstrual age. Infants could have had the dose split at the discretion of the responsible clinical team. All other aspects of care, including the timing of the commencement of enteral feeds and the type of milk feed used were as per local policy, practice, and discretion. Administration of the trial IMP might have been stopped temporarily. Missed doses did not necessitate the removal of an infant from the trial. Data continued to be collected as per protocol if the trial medication was stopped. As participants were inpatients in a neonatal unit they were monitored continuously, and samples were only collected when indicated

Panel 1: Definition of microbiologically confirmed late-onset infection*

Microbiological culture of potentially pathogenic bacteria (including coagulase-negative staphylococci species, but excluding probable skin contaminants such as diphtheroids, micrococci, propionibacteria, or a mixed flora) or fungi from blood or cerebrospinal fluid sampled aseptically more than 72 h after birth, and treatment, or clinician intention to treat, for 5 days or more with intravenous antibiotics (excluding antimicrobial prophylaxis) after investigation was done. If the infant died or was discharged or transferred before the completion of 5 days of antibiotics, this condition would still be met if the intention was to treat for at least 5 days.

*Adapted from the UK Neonatal Infection Surveillance Network case-definition⁷

Panel 2: Definition of clinically suspected late-onset infection*

Absence of positive microbiological culture, or culture of a mixed microbial flora or of probable skin contaminants (ie, diphtheroids, micrococci, propionibacteria) only, and treatment or clinician intention to treat for 5 days or more with intravenous antibiotics (excluding antimicrobial prophylaxis) after the investigation was undertaken for an infant who presents at least 3 of the following clinical or laboratory features of invasive infection:

- increase in oxygen requirement or ventilatory support
- increase in frequency of episodes of bradycardia or apnoea
- temperature instability
- ileus or enteral feeds intolerance or abdominal distention
- reduced urine output to less than 1 mL/kg per h
- impaired peripheral perfusion (capillary refill time longer than 3 seconds, skin mottling or core-peripheral temperature gap greater than 2°C)
- hypotension (clinician-defined as needing volume or inotrope support)
- irritability, lethargy, or hypotonia (clinician-defined)
- increase in serum C-reactive protein concentrations to more than 15 mg/L or in procalcitonin concentrations to 2 ng/mL or more
- white blood cells count smaller than 4×10^9 /L or greater than 20×10^9 /L
- platelet count less than 100×10^9 /L
- glucose intolerance (blood glucose smaller than 40 mg/dL or greater than 180 mg/dL)
- metabolic acidosis (base excess less than -10 mmol/L or lactate concentration greater than 2 mmol/L)

*Adapted from the European Medicines Agency consensus criteria and predictive model¹⁸

For the British Association of Perinatal Medicine categories of care see <https://www.bapm.org/resources/categories-care-2011>

clinically. When an infant completed the course of treatment, any unused IMP pots were accounted for and destroyed.

Outcomes

The primary outcome was microbiologically confirmed (panel 1) or clinically suspected (panel 2) late-onset infection, from trial entry until hospital discharge. Primary outcome was first assessed locally and then centrally by a blinded end-point review committee.

Secondary outcomes were microbiologically confirmed infection; all-cause mortality before hospital discharge; necrotising enterocolitis (Bell's stage 2 or 3);¹⁹ severe retinopathy of prematurity treated medically or surgically;²⁰ bronchopulmonary dysplasia for which the infant is still receiving mechanical ventilator support or supplemental oxygen at 36 weeks' post-menstrual age;²¹ a composite of invasive infection, major morbidity

(necrotising enterocolitis, retinopathy of prematurity, or bronchopulmonary dysplasia), and mortality; total number of days of administration of antimicrobials per infant from trial entry until 34 weeks' postmenstrual age; total length of stay until discharge home; and length of stay in intensive care, high dependency care, and special care, as defined by the British Association of Perinatal Medicine.

An end-point review committee masked to participant allocation reviewed all case report forms reporting episodes of late-onset infection or necrotising enterocolitis. Two members independently assessed adherence to case-definitions and resolved any discrepancies by discussion or referral to a third committee member. Persisting uncertainties were discussed with the site principal investigator or research nurse or both.

Some adverse events were expected (necrotising enterocolitis, bronchopulmonary dysplasia, retinopathy of prematurity, death) because of the nature of the participant population and their routine care and treatment. No adverse drug reactions were expected from bovine lactoferrin. Expected serious adverse events were recorded on the case report forms. All other serious adverse events were reported by trial sites to the NPEU Clinical Trials Unit (CTU) within 24 h of being recognised. A standard operating procedure outlining the reporting procedure for clinicians was provided with the serious adverse events form and in the trial handbook. The NPEU CTU processed and reported the event as specified in its own standard operating procedures. All serious adverse events were reviewed by the independent Data Monitoring Committee (DMC) at regular intervals throughout the trial. The chief investigator notified all investigators of information that could affect the safety of participants.

Suspected unexpected serious adverse reactions (SUSAR) were reported to the MHRA and the approving Research Ethics Committee within 7 days, if life threatening, and within 15 days for other SUSARs. A copy of the SUSAR form was forwarded to the Chair of the DMC. The Chair was provided with details of all previous SUSARs with their unmasked allocation. The chief investigator informed all investigators of any issues raised in a SUSAR that could affect the safety of participants.

The chief investigator submitted annually or on request a development safety update report to the competent authority, the approving research ethics committee, and the sponsor.

Post-hoc analyses assessed differential effects depending on the infecting microorganism identified for the microbiologically confirmed late-onset infection outcome, and differential effects for the primary outcome depending on whether infants had or had not received probiotics as part of their routine care.

Statistical analysis

The sample size estimate was informed by a range of plausible primary outcome control event rates (CER)

from 18% to 24%.^{2,3} In summary, with 90% power and two-sided 5% significance, to detect an absolute risk reduction of 5–5.8% (relative risk reduction of 24–28%) would require a total of up to 2200 participants if the CER was 18%, 2070 if the CER was 21%, and 2076 if the CER was 24%. This target sample size of 2200 allowed for an anticipated loss to follow-up of up to 5%.

Characteristics at randomisation were summarised with counts (percentages) for categorical variables, mean (SD) for normally distributed continuous variables, or median (IQR) for other continuous variables.

Primary and secondary outcomes for participants were analysed in the groups to which they were assigned excluding those who withdrew consent for their data to be used or for whom consent was not obtained. Safety data were collected for all participants randomly assigned to groups. Comparative analyses calculated the relative risk ratio (RR) with 95% CI for the primary outcome (99% CIs for all other dichotomous outcomes), the mean difference (99% CI) for normally distributed continuous outcomes, or the median difference (99% CI) for skewed continuous variables.

The groups were compared using regression analysis adjusting for the minimisation factors (ie, collaborating hospital, sex, gestational age at birth, and single or multiple birth) to account for the correlation between treatment groups introduced by balancing the randomisation. For all binary outcomes, we used mixed effect Poisson models with robust standard errors. Centre was fitted as a random effect and mother's identification nested within this to take account of clustering within centre and within multiples. The other minimisation factors were fitted as a fixed effect with sex and multiplicity of birth included as binary variables and gestational age modelled as a continuous variable. For all length-of-stay outcomes and the number of days of administration of antimicrobials, we used quantile regression with sex and multiplicity of birth included as binary variables and gestational age modelled as a continuous variable. The crude unadjusted and adjusted estimates were calculated with the primary inference to be based on the adjusted analysis.

The consistency of the effect of lactoferrin supplementation on the primary outcome across specific subgroups of infants was assessed using the statistical test of interaction. Prespecified subgroups were number of completed weeks' gestation at birth, and type of milk given to the participating infants during the trial period (human breast milk versus formula versus both human milk and formula).

The trial statisticians produced reports for the DMC and Trial Steering Committee (TSC). Data quality issues were reported to data management staff and investigated when appropriate or included in routine data validation checks, or both. The TSC and DMC meetings provided opportunities for external, independent review of summary data, with additional feedback on potential

data quality issues being incorporated into ongoing data quality checks.

The analyses were done using Stata/SE version 13.1 for Windows.²²

This trial is registered with the International Standard Randomised Controlled Trial, number 88261002.

Role of the funding source

The funder provided advice and support and monitored study progress but did not have a role in study design or data collection, analysis, and interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

2203 participants in 37 neonatal units were recruited between May 7, 2014, and Sept 28, 2017 (figure 1). 1099 infants were randomly assigned to the lactoferrin group and 1104 infants to the placebo group. Consent was not confirmed for one infant in the lactoferrin group and withdrawn for three infants in the placebo group; these infants were not included in the analysis of outcomes at discharge. Therefore, in total, 1098 infants in the lactoferrin group and 1101 in the placebo group were included in the modified intention-to-treat analyses. Data were not available for five infants in the lactoferrin group and 12 infants in the control group; hence, 1093 infants in the lactoferrin group and 1089 in the placebo group were included in the primary and secondary outcome analyses. Baseline characteristics and other demographic features of participating infants in the two treatment allocation groups are presented in table 1.

35 (2%) infants discontinued the intervention early, 20 (2%) of 1098 in the lactoferrin group and 15 (1%) of 1101 in the sucrose group (appendix). Parental consent remained for data collection for intention-to-treat analyses for 32 (91%) of the 35 infants.

Adherence was high for infants who continued to receive the IMP. The median percentage of days when an IMP dose was not given or not recorded was 4% (IQR 0 to 18.18 for the lactoferrin group; IQR 0 to 16.22 for the control group) in both treatment groups. The median difference between expected dose and actual dose per day was 7 mg/kg per day (IQR: –9 to 0 for the lactoferrin group; IQR –27 to 0 for the control group) in both groups, and was 1 mg/kg per day (IQR –11 to 0; lactoferrin group) or 2 mg/kg per day (IQR –11 to 0; control group) if the days in which enteral feeds were stopped or withheld for more than 4 h were excluded.

The estimates of effect for the primary and secondary outcomes are presented in table 2.

Data were available for 2182 infants (99.0%; case report forms for primary or secondary outcomes were incomplete or non-verifiable for missing infants). 316 (28.9%) of 1093 infants in the lactoferrin group acquired a microbiologically confirmed or clinically

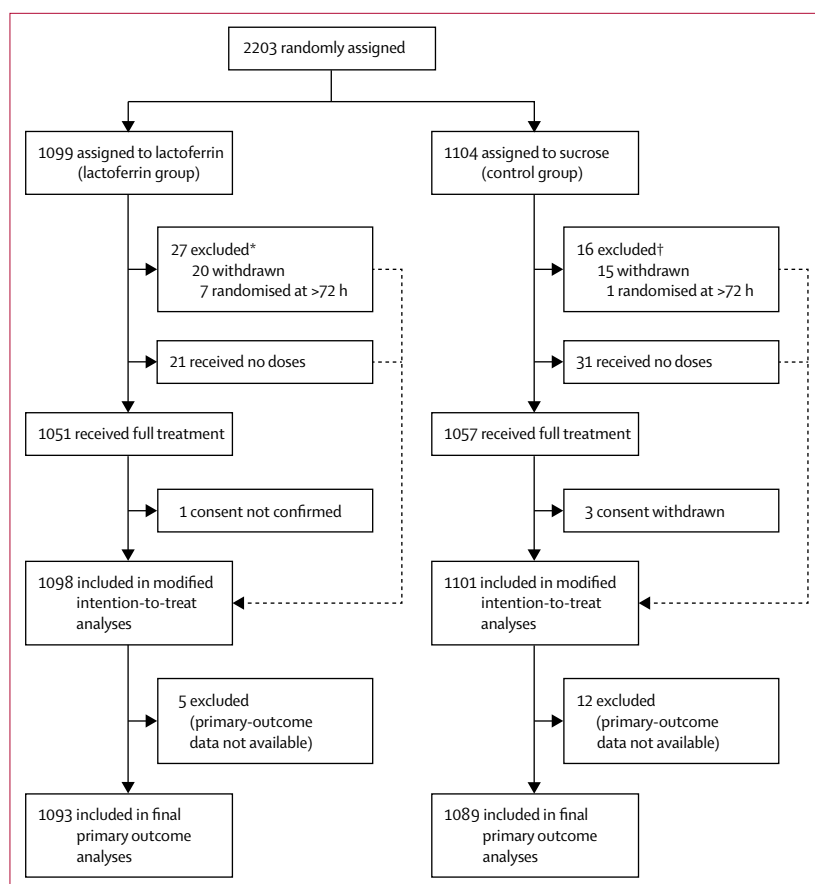


Figure 1: Trial profile

Number of infants assessed for eligibility and excluded before randomisation is not available. *Consent to use data remained for 20 infants and, when available, data were included in the analysis. †Consent to use data remained for 12 infants and, when available, data were included in the analysis.

suspected late-onset infection versus 334 (30.7%) of 1089 in the control group (table 2). The adjusted RR was 0.95 (95% CI 0.86–1.04). Subgroup analyses did not show any significant interactions for completed weeks' gestation at birth or type of enteral milk received (figure 2).

Post-hoc analyses did not show any effects depending on the infecting microorganism identified for the microbiologically confirmed late-onset infection outcome, nor did they appear to show any difference in primary outcome by trial group depending on whether infants had received probiotics as part of routine care or not (table 3).

There were no significant differences in secondary outcomes (table 2): microbiologically confirmed infection (RR 1.05 [99% CI 0.87–1.26]); all-cause mortality (1.05 [0.66–1.68]); necrotising enterocolitis (1.13 [0.68–1.89]); retinopathy of prematurity (0.89 [0.62–1.28]); bronchopulmonary dysplasia (RR 1.01 [0.90–1.13]); or a composite of infection, major morbidity, and mortality (1.01 [0.94–1.08]). There were no differences in the number of days of administration of antimicrobials until 34 weeks' postmenstrual age, length of stay in hospital, or

Number of centres*	37	37
Male sex*	575/1098 (52%)	578/1099 (53%)
Infant age at randomisation in days, median (IQR)	2 (2–3)	2 (2–3)
Birthweight (g), mean (SD)	1125.9 (356.2)	1143.3 (367.1)
<500	8/1098 (1%)	7/1101 (1%)
500–749	172/1098 (16%)	172/1101 (16%)
750–999	254/1098 (23%)	244/1101 (22%)
1000–1249	268/1098 (24%)	255/1101 (23%)
1250–1499	199/1098 (18%)	199/1101 (18%)
≥1500	197/1098 (18%)	224/1101 (20%)
Birthweight <10th centile for gestational age	175/1097 (16%)	177/1098 (16%)
Gestation at delivery (completed weeks),* median (IQR)	29 (27–30)	29 (27–30)
<23	1/1098 (<1%)	1/1101 (<1%)
23 + 0 to 23 + 6	33/1098 (3%)	31/1101 (3%)
24 + 0 to 24 + 6	73/1098 (7%)	76/1101 (7%)
25 + 0 to 25 + 6	73/1098 (7%)	73/1101 (7%)
26 + 0 to 27 + 6	227/1098 (21%)	221/1101 (20%)
28 + 0 to 29 + 6	315/1098 (29%)	319/1101 (29%)
30 + 0 to 31 + 6	376/1098 (34%)	380/1101 (35%)
Mother's age at randomisation in years, mean (SD)	30.3 (6.1)	30.4 (6.0)
Multiple pregnancy*	350/1098 (32%)	346/1101 (31%)
Caesarean section delivery	635/1098 (58%)	616/1101 (56%)
Membranes ruptured before labour	422/1093 (39%)	428/1097 (39%)
Membranes ruptured >24 h before delivery	286/1092 (26%)	264/1096 (24%)
Mother received antenatal corticosteroids	998/1093 (91%)	997/1099 (91%)
Infant heart rate >100 bpm at 5 min from birth	995/1090 (91%)	1010/1093 (92%)
Infant temperature on admission (°C), mean (SD)	36.9 (0.7)	37 (0.7)
Infant worst base excess within first 24 h of birth, mean (SD)	−6.2 (3.9)	−6.3 (3.8)
Infant ventilated via endotracheal tube at randomisation	338/1098 (31%)	357/1101 (32%)
Infant had absent or reverse end diastolic flow in the umbilical artery antenatally	134/1079 (12%)	130/1081 (12%)

Unless otherwise stated, data are n/N (%); when N is not equal to the total number of infants in the group it means that data are missing for some of the infants. BPM=beats per minute. *Minimisation factor.

Table 1: Infant and maternal baseline characteristics

length of stay in intensive care, high dependency care, or special care levels.

There were 16 serious adverse events (1.5%) reported for infants in the lactoferrin group (six [0.5%] severe; n=1093), and ten (0.9%) for the sucrose group (three [0.3%] severe; n=1089; table 4). Two serious adverse events—ie, one case of blood in stool (expected) and one death after intestinal perforation probably

	Lactoferrin group (n=1098)	Control group (n=1101)	Unadjusted risk ratio or median difference (95% CI or 99% CI)*†	Adjusted risk ratio or median difference (95% CI or 99% CI)*‡	p value§
Microbiologically confirmed or clinically suspected late-onset infection	316/1093 (29%)	334/1089 (31%)	0.94 (0.83 to 1.07)	0.95 (0.86 to 1.04)	0.233
Microbiologically confirmed late-onset infection	190/1093 (17%)	180/1089 (17%)	1.05 (0.82 to 1.34)	1.05 (0.87 to 1.26)	0.490
All-cause mortality	71/1076 (7%)	68/1076 (6%)	1.04 (0.69 to 1.59)	1.05 (0.66 to 1.68)	0.782
NEC (Bell stage II or III)	63/1085 (6%)	56/1084 (5%)	1.12 (0.71 to 1.77)	1.13 (0.68 to 1.89)	0.538
Severe ROP treated medically or surgically	64/1080 (6%)	72/1080 (7%)	0.89 (0.58 to 1.35)	0.89 (0.62 to 1.28)	0.420
BPD at 36 weeks' postmenstrual age	358/1023 (35%)	355/1027 (35%)	1.01 (0.87 to 1.18)	1.01 (0.90 to 1.13)	0.867
Died before 36 weeks' postmenstrual age	64	60
Infection, NEC, ROP, BPD, or mortality	525/1092 (48%)	521/1094 (48%)	1.01 (0.90 to 1.13)	1.01 (0.94 to 1.08)	0.743
Total number of days of administration of antimicrobials from commencement of investigational medicinal product until 34 weeks' postmenstrual age, median (IQR)	2 (0 to 8)	3 (0 to 8)	0 (0 to 0)	0 (-1 to 1)	0.625
Length of hospital stay (days) to discharge, median (IQR)	59 (40 to 85)	58 (40 to 84)	1 (-2 to 4)	1 (-1 to 3)	0.446
Days in level 1 (intensive) care, median (IQR)	8 (4 to 16)	8 (4 to 16)	0 (-1 to 1)	0 (-1 to 1)	0.963
Days in level 2 (high dependency) care, median (IQR)	10 (3 to 30)	9 (3 to 29)	0 (-1 to 1)	1 (-1 to 3)	0.420
Days in level 3 (special) care, median (IQR)	29 (21 to 39)	30 (22 to 39)	-1 (-2 to 1)	-1 (-3 to 1)	0.216

Unless otherwise stated, data are n/N (%); when N is not equal to the total number of infants in the group it means that data are missing for some of the infants. NEC=necrotising enterocolitis. BPD=bronchopulmonary dysplasia. ROP=retinopathy of prematurity. *Risk ratios for binary outcomes and median differences for continuous outcomes. †95% CI for microbiologically confirmed or clinically suspected late-onset invasive infection, 99% CI for all other outcomes. ‡Adjusted for minimisation factors (ie, collaborating hospital, sex, gestational age at birth, and single or multiple birth). §p value for testing whether adjusted risk ratio is equal to 1 or adjusted median difference is equal to 0.

Table 2: Primary and secondary outcomes

associated with necrotising enterocolitis (SUSAR)—both in the lactoferrin group, were assessed as being possibly related to the trial intervention. The remaining 24 serious adverse events were considered to be unrelated to the trial intervention.

Discussion

Our data indicate that enteral lactoferrin supplementation (150 mg/kg per day until 34 weeks' postmenstrual age) does not reduce the risk of late-onset infection, other morbidity, or mortality in very preterm infants. This contradicts previous trial findings. The 2017 Cochrane review¹⁵ includes six RCTs, and meta-analyses suggest substantial reductions in late-onset infection and necrotising enterocolitis associated with lactoferrin supplementation in very preterm infants. These trials, however, were small and some contained design and methodological weaknesses that might have introduced performance and detection biases resulting in over-estimation of the treatment effects. Given these concerns, the Cochrane review graded the evidence for key outcomes as low quality and concluded that data from large, methodologically-rigorous RCTs were needed to generate evidence of sufficient validity to inform policy and practice.

The ELFIN trial provides such evidence. We used practices to limit bias, such as central web-based randomisation for allocation concealment; masking of parents, caregivers, and investigators to the group allocation; and complete follow-up and assessment of the trial cohort with intention-to-treat analyses based on a prespecified statistical-analysis plan. The trial

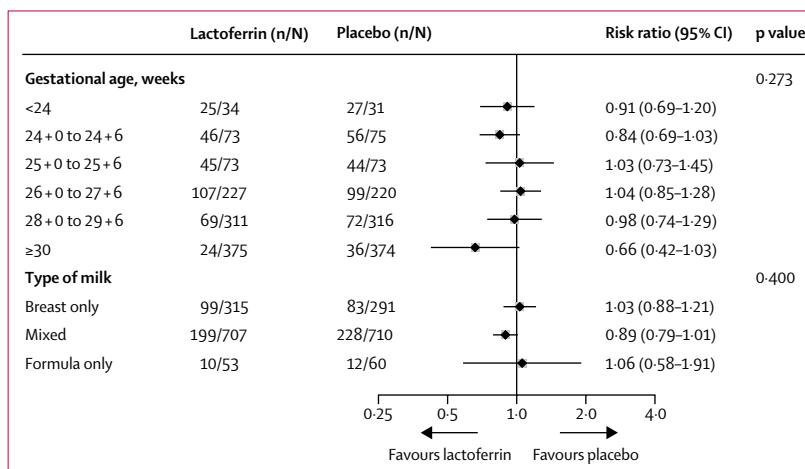


Figure 2: Subgroup analyses for confirmed or suspected late-onset invasive infection
p values are for statistical interactions. n=cases of late-onset invasive infection. N=group size.

achieved recruitment of 2203 participants as per protocol, which was based on an a priori sample-size estimation. Demographic and prognostic characteristics were well balanced at randomisation. Interim analyses by the trial's independent DMC used criteria to minimise the chances of spurious findings due to data fluctuations before a sufficient sample size was achieved.^{23,24} Adherence to the intervention was high, the proportion of protocol violations was low, and outcome data were available for more than 99% of the cohort. Event prevalence for the outcomes was broadly similar to that anticipated.^{2,3} Consequently, the trial had sufficient power and validity to detect reliably modest

	Lactoferrin group (n=1098)	Control group (n=1101)
Microbiologically confirmed late-onset invasive infection from trial entry until hospital discharge	190/1093 (17.4%)	180/1089 (16.5%)
At least one Gram-positive organism confirmed	153/1093 (14.0%)	147/1089 (13.5%)
At least one CoNS group organism	122/1093 (11.2%)	111/1089 (10.2%)
At least one Gram-negative organism confirmed	46/1093 (4.2%)	39/1089 (3.6%)
At least one fungal organism confirmed	3/1093 (0.3%)	2/1089 (0.2%)
At least one other organism confirmed	3/1093 (0.3%)	2/1089 (0.2%)
Any record of probiotics being given		
Yes	99/354 (28.0%)	97/329 (29.5%)
No	208/728 (28.6%)	227/749 (30.3%)

Data for late-onset infection by classification of microorganism are n/N (%); when N is not equal to the total number of infants in the group it means that data are missing for some of the infants. Data for exposure to probiotics are number of infants with microbiologically confirmed or clinically suspected late-onset infection/number of infants who were given (or not) probiotics (%). CoNS=coagulase-negative staphylococcus.

Table 3: Microbiologically confirmed late-onset infection by classification of microorganism (appendix) and microbiologically confirmed or clinically suspected late-onset infection from trial entry until hospital discharge by exposure to probiotics

yet important effects on the risk of late-onset infection and other morbidity.

The ELFIN trial enrolled more than twice as many infants than had participated in all of the existing trials combined and generated more precise estimates of effect size than were previously available. Our relative risk estimate (0.95, 95% CI 0.86–1.04) for the primary outcome is consistent with a possible risk reduction of up to 14% and an increase in risk of up to 4%. These estimates were consistent across gestational age bands, making it unlikely that bovine lactoferrin has any important benefits for extremely preterm infants (ie, born before 28 weeks' gestation) who have an increased risk of infection. Similarly, although plausible that lactoferrin might have had different effects in infants with low levels of exposure to the immuno-protective factors present in human milk, we did not show any interaction with the type of enteral milk feeds received during the trial period (human milk, formula, or both).

	Age (days)	Brief description of event	Severity	Related to trial
Lactoferrin group (n=1098)				
1	12	Meconium ileus following one dose of investigational medicinal product; resolved with laparotomy, no bowel removed	Moderate	No
2	30	Two episodes of clinical seizures, resolved with brief course of anticonvulsant	Moderate	No
3	59	Cluster of seizures, probably related to severe Gram-negative bacteraemia and sepsis (ultimately fatal)	Severe	No
4	12	Episode of supraventricular tachycardia, resolved with adenosine and propranolol	Mild	No
5	49	Metabolic acidosis (likely renal tubular acidosis), resolved with sodium bicarbonate	Severe	No
6	20	Episode of supraventricular tachycardia, resolved with face cooling	Mild	No
7	19	Suspected necrotising enterocolitis	Moderate	No
8	18	Cluster of clinical seizures, resolved with magnesium sulphate and course of phenobarbitone	Mild	No
9	81	Infective exacerbation of chronic lung disease, resolved with antibiotics and corticosteroids	Severe	No
10	17	Large inferior vena cava thrombus	Moderate	No
11	68	Acute airway obstruction, resolved with respiratory support	Severe	No
12	44	Aspiration pneumonia resolved with respiratory support	Severe	No
13	21	Blood in stool, unknown cause, resolved spontaneously	Moderate	Possibly (expected)
14	19	Haemolytic anaemia, unknown cause, resolved spontaneously	Mild	No
15	10	Death following intestinal perforation secondary to necrotising enterocolitis	Severe	Possibly (SUSAR)
16	27	Death attributed to Gram-negative bacteraemia	Severe	No
Control group (n=1101)				
1	61	Rib fracture secondary to osteopenia of prematurity, resolved with supportive care and nutrient supplementation	Moderate	No
2	50	Superior sagittal sinus non-occlusive thrombus, resolved with heparin (6 weeks of treatment)	Moderate	No
3	48	Hyperammonaemia, unknown cause, resolved with course of sodium benzoate	Moderate	No
4	36	Death attributed to infection and sepsis	Severe	No
5	24	Episode of tachycardia and ectopic beats, resolved with face cooling and reduction in caffeine dose	Mild	No
6	37	Death secondary to exacerbation of chronic lung disease (severe bronchopulmonary dysplasia)	Severe	No
7	26	Death attributed to severe bronchopulmonary dysplasia	Severe	No
8	57	S aureus bacteraemia and osteomyelitis, resolved with antibiotics	Moderate	No
9	22	Episode of supraventricular tachycardia, resolved with adenosine	Moderate	No
10	6	Episode of supraventricular tachycardia, resolved with carotid massage and adenosine	Mild	No

Each event affected one infant. SUSAR=suspected unexpected serious adverse reactions.

Table 4: List of serious adverse events reported by randomisation group

The largest previous trial²⁶ of enteral lactoferrin supplementation, in which 331 very low-birthweight infants in a neonatal unit in Italy participated, showed a relative risk reduction of 66% (risk ratio 0·34, 95% CI 0·17–0·70) for late-onset infection. The participants and the intervention were broadly similar to the ELFIN trial, as were enteral-feeding practices, including receipt of human breast milk versus formula. However, key differences in the epidemiology of late-onset infection, as well as in infection-prevention practices and exposure to other interventions, might have contributed to the difference in effects size estimates shown in the two trials. Notably, the prevalence of invasive fungal infection was very high in the Italian trial (7·7% of the control group) and a substantial proportion of the overall effect on reducing late-onset invasive infection was due to the effect on preventing invasive fungal infection. By contrast, the overall prevalence of late-onset fungal infection was low in the ELFIN trial (five episodes in total), consistent with that reported in UK surveillance studies.²

A limitation of our study was that pre-specified primary outcome included both clinically suspected and microbiologically confirmed late-onset infection. We took this pragmatic approach because of concerns about the diagnostic accuracy of microbiological culture of blood in this population.²⁵ Standard microbiological culture might not detect cases of bacteraemia or fungaemia if an insufficient volume of the infant's blood is incubated (ie, false-negative result). Conversely, microbiological cultures can also generate false-positive results if blood sampling techniques allow entry of contaminating microorganisms (typically from the infant's skin). To mitigate these potential sources of bias, we used an established consensus case definition that required additional evidence of infection (clinical signs or biomarkers) and mandated that clinicians indicate an intention to treat the infant with antibiotics or antifungals for at least 5 days.²

Given that a postulated mechanism of action of lactoferrin is to reduce bowel translocation of enteric pathogens, we assessed post-hoc whether invasive infections with particular groups of enteric organisms were reduced. We did not find any evidence that lactoferrin supplementation affected the risk of late-onset infection with different groups of infecting microorganism including Gram-negative bacteria (mainly *Escherichia coli* and other enterobacteriaceae).

In a post-hoc subgroup analysis of infants who had or had not received routine probiotic supplementation during the trial period, we did not show any difference in the effect of lactoferrin on the risk of late-onset infection. A previous trial²⁶ and the 2017 Cochrane review¹⁵ have suggested that combining supplementation of lactoferrin with the probiotic microorganism *Lactobacillus rhamnosus* GG was associated with a greater reduction in the risk of late-onset infection and necrotising enterocolitis than was lactoferrin supplementation alone. This raises the

possibility that the immunoprotective and prebiotic properties of lactoferrin might act synergistically with probiotic supplementation.²⁷ Although the ELFIN trial did not show any evidence of differential effects depending upon whether infants had received probiotics during the trial period, the data are not sufficient to exclude the possibility that such prebiotic–probiotic synergism exists. A 2017 large cluster RCT²⁸ in India has suggested that prophylactic administration of an oral synbiotic (prebiotic fructo-oligosaccharide combined with probiotic *Lactobacillus plantarum*) reduces infection and mortality in late-preterm or term newborn infants. We are doing a mechanistic study²⁷ in a subgroup of ELFIN trial participants to analyse whether and how lactoferrin supplementation affects the intestinal microbiome and metabolite profile. The study, which is ongoing, will explore changes in microbiomic and metabolomic patterns preceding disease onset, including necrotising enterocolitis and late-onset infection.

Estimates for the secondary outcomes indicated consistently that lactoferrin supplementation does not have important effects on the risk of major morbidities. We prespecified an analysis of the effect on a composite of infection, necrotising enterocolitis, bronchopulmonary dysplasia, retinopathy of prematurity, or death. The adjusted risk ratio point estimate was 1·01 (95% CI 0·94–1·08), which is consistent with a possible risk reduction of up to 6% and a possible increase in risk of up to 8%. We plan to increase the precision of the estimates of effect on rare secondary outcomes by combining these data in a meta-analysis with other trials, including a recently completed Australasian RCT of bovine-lactoferrin supplementation for very low-birthweight infants (Lactoferrin Infant Feeding Trial; unpublished).

Since late-onset infection and necrotising enterocolitis are the major reasons for receipt of invasive interventions and high levels of care in very preterm infants, it is not surprising that we did not find any effects on the degree of exposure to antimicrobial drugs, on the duration of hospitalisation, or on stay in intensive or high-dependency care settings. Given that the ELFIN trial did not show any differences between groups in the risk of morbidity or on levels of care received, we did not undertake further analyses of health-care costs as had been proposed in our approved funding application and trial protocol. We did not do a within-trial health economic analysis or use these data in a model to explore long-term family and health-service costs since these are mainly driven by the consequences of infection and other morbidity during the initial hospitalisation. Without evidence of clinical effectiveness on these infant-important outcomes, we considered a cost-effectiveness analysis of lactoferrin supplementation to be futile.²⁹

We do not plan to apply for permission and funding to assess longer-term outcomes of trial participants. We specified in our protocol that if the trial did not detect significant or clinically important differences in the

For the Lactoferrin Infant Feeding Trial see www.anzctr.org.au/ACTRN12611000247976.aspx

in-hospital outcomes, then follow-up would not be done since any between-group differences in growth and neurodevelopmental outcomes are predicated largely on differences in the prevalence of late-onset infections, necrotising enterocolitis, and associated morbidities.⁴ Since these were not shown, there is no longer an impelling rationale for expecting lactoferrin supplementation to have an effect on long-term growth or development.

The ELFIN trial findings are likely to be applicable in the UK and internationally. The trial population was representative of very preterm infants cared for within health-care facilities in well resourced health services and included a substantial proportion of extremely preterm infants and of infants with other putative risk factors for neonatal morbidity. Overall, about 30% of participants acquired a microbiologically confirmed or clinically suspected late-onset infection, and about 17% in total had a microbiologically confirmed infection, consistent with prevalence values reported from cohort studies and other RCTs. Similarly, the prevalence of necrotising enterocolitis (about 5%) was similar to that reported in large, population-based surveillance and cohort studies and RCTs.³⁰

In conclusion, the ELFIN trial does not support the routine use of enteral bovine lactoferrin supplementation to prevent late-onset infection or other morbidity or mortality in very preterm infants. Research efforts should continue to investigate the aetiology, epidemiology, and pathogenesis of late-onset infection and related morbidities, and to develop, refine, and assess other interventions that could prevent or reduce adverse acute and long-term consequences for very preterm infants and their families.

ELFIN trial investigators group

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Contributors

JG is the trial manager; PJ the trial research nurse; MV the administrator and data coordinator; UB the senior trials manager; EJ the NPEU Clinical Trial Unit director; AK the head of trials programming; DM the senior trials programmer; MP the infants and parents advocate;

WMCg the chief investigator; PH a co-investigator and chair of the blinded-endpoint review committee; LL and CP are the trial statisticians; JB, JD, NE, and SO are coinvestigators. JB, NE, PH, EJ, LL, WMCg, and SO designed the study. JG, PJ, MV, UB, AK, LL, DM, PH, and WMCg collected and managed the data. LL, CP, EJ, WMCg, and PH analysed the data. JB, NE, MP, PH, EJ, WMCg, and SO interpreted the data. JG, EJ, LL, CP, and WMCg wrote the Article. All authors approved the final draft of the manuscript.

Declaration of interests

EJ and JD are members of the National Institute for Health Research—Health Technology Assessment (NIHR HTA) General Board; WMCg is a member of the NIHR HTA Commissioning Board and the HTA Journals Library editorial board. PH, WMCg, EJ, LL report receipt of funding from NIHR, outside the submitted work. NE reports grants from Prolacta Biosciences US and Danone Early Life Nutrition, personal fees from Nestle Nutrition Institute, and personal fees from Baxter, outside the submitted work. All other authors have nothing to declare.

Acknowledgments

The ELFIN trial was funded by the National Institute for Health Research—Health Technology Assessment Programme (10/57/49). We thank all the parents of participating infants and all staff and carers in recruiting and continuing care sites. We thank the members of the independent Data Monitoring Committee and Trial Steering Committee; the Newcastle Specials Pharmacy team; Bliss; Tatua Dairy Co-operative, New Zealand; and the administrative and support colleagues at the NPEU CTU.

References

- Duley L, Uhm S, Oliver S. Preterm Birth Priority Setting Partnership Steering Group. Top 15 UK research priorities for preterm birth. *Lancet* 2014; **383**: 2041–42.
- Cailles B, Kortsalioudaki C, Buttery J, et al. Epidemiology of UK neonatal infections: the neonatal infection surveillance network. *Arch Dis Child Fetal Neonatal Ed* 2017; **103**: F547–53.
- Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. *Lancet* 2017; **390**: 1770–80.
- Bassler D, Stoll BJ, Schmidt B, et al. Using a count of neonatal morbidities to predict poor outcome in extremely low birth weight infants: added role of neonatal infection. *Pediatrics* 2009; **123**: 313–18.
- Payne NR, Carpenter JH, Badger GJ, Horbar JD, Rogowski J. Marginal increase in cost and excess length of stay associated with nosocomial bloodstream infections in surviving very low birth weight infants. *Pediatrics* 2004; **114**: 348–55.
- Embleton ND, Berrington JE, McGuire W, Stewart CJ, Cummings SP. Lactoferrin: antimicrobial activity and therapeutic potential. *Semin Fetal Neonatal Med* 2013; **18**: 143–49.
- Legrand D. Overview of lactoferrin as a natural immune modulator. *J Pediatr* 2016; **173**: S10–15.
- Raghuveer TS, McGuire EM, Martin SM, et al. Lactoferrin in the preterm infants' diet attenuates iron-induced oxidation products. *Pediatr Res* 2002; **52**: 964–72.
- Cuenca AG, Wynn JL, Moldawer LL, Levy O. Role of innate immunity in neonatal infection. *Am J Perinatol* 2013; **30**: 105–12.
- Lingappan K, Arunachalam A, Pammi M. Lactoferrin and the newborn: current perspectives. *Expert Rev Anti Infect Ther* 2013; **11**: 695–707.
- Vorland LH, Ulvatne H, Andersen J, et al. Lactoferricin of bovine origin is more active than lactoferricins of human, murine and caprine origin. *Scand J Infect Dis* 1998; **30**: 513–17.
- Office of Food Additive Safety. GRAS notification. Food and Drug Administration. <https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=669> (accessed June 23, 2018).
- Appel MJ, van Veen HA, Vietsch H, et al. Sub-chronic (13-week) oral toxicity study in rats with recombinant human lactoferrin produced in the milk of transgenic cows. *Food Chem Toxicol* 2006; **44**: 964–73.
- EFSA Panel on Dietetic Products, Nutrition and Allergies. Scientific Opinion on bovine lactoferrin. *EFSA J* 2012; **10**: 2701.
- Pammi M, Suresh G. Enteral lactoferrin supplementation for prevention of sepsis and necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2017; **6**: CD007137.

- 16 ELFIN Trial Investigators Group. Lactoferrin immunoprophylaxis for very preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2013; **98**: F2–4.
- 17 The ELFIN Trial Investigators Group. Summary protocol for a multi-centre randomised controlled trial of enteral lactoferrin supplementation in newborn very preterm infants (ELFIN). *Neonatology* 2018; **114**: 142–48.
- 18 Modi N, Doré CJ, Saraswatula A, et al. A case definition for national and international neonatal bloodstream infection surveillance. *Arch Dis Child Fetal Neonatal Ed* 2009; **94**: F8–12.
- 19 Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am* 1986; **33**: 179–201.
- 20 ICROP. An International Classification of Retinopathy of Prematurity. *Pediatrics* 1984; **74**: 127–33.
- 21 Ehrenkranz RA, Walsh MC, Vohr BR, et al. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. *Pediatrics* 2005; **116**: 1353–60.
- 22 StataCorp. Stata statistical software: release 13. College Station, TX: StataCorp LP, 2013.
- 23 DAMOCLES Study Group, NHS Health Technology Assessment Programme. A proposed charter for clinical trial data monitoring committees: helping them to do their job well. *Lancet* 2005; **365**: 711–22.
- 24 Ioannidis JP. Why most discovered true associations are inflated. *Epidemiology* 2008; **19**: 640–48.
- 25 Oeser C, Lutsar I, Metsvaht T, Turner MA, Heath PT, Sharland M. Clinical trials in neonatal sepsis. *J Antimicrob Chemother* 2013; **68**: 2733–45.
- 26 Manzoni P, Rinaldi M, Cattani S, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. *JAMA* 2009; **302**: 1421–28.
- 27 Embleton ND, Berrington JE, Dorling J, et al. Mechanisms affecting the gut of preterm infants in enteral feeding trials. *Front Nutr* 2017; **4**: 14.
- 28 Panigrahi P, Parida S, Nanda NC, et al. A randomized symbiotic trial to prevent sepsis among infants in rural India. *Nature* 2017; **548**: 407–12.
- 29 Sculpher MJ, Pang FS, Manca A, et al. Generalisability in economic evaluation studies in healthcare: a review and case studies. *Health Technol Assess* 2004; **8**: 1–192.
- 30 Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: a systematic review. *Arch Dis Child Fetal Neonatal Ed* 2018; **103**: F182–89.